PHYSICAL MAPPING OF A MAJOR QTL CONDITIONING COMMON BACTERIAL BLIGHT ON CHROMOSOME 1 IN COMMON BEAN

Shuyu Liu, Kangfu Yu, Margaret Haffner, and Soon J. Park
Agriculture Agri-Food Canada, Greenhouse and Processing Crops Research Center, Harrow ON N0R 1G0, Canada. Yuk@agr.gc.ca

Abstract
A major QTL conditioning common bacterial blight (CBB) resistance in bean lines HR45 and HR67 was derived from XAN159 whose resistance was inherited from tepary bean line PI319443. Four markers have been found tightly linked to this major CBB resistance QTL. A BAC library was constructed from high molecular weight DNA of HR45 and has 5.7-fold bean genome coverage. We screened BAC pools using these markers. Two to eight BAC clones were identified from each marker. Two clones were found to have both markers PV-tttc001 and STS183. Preliminary contigs covering this major QTL were constructed. This is the first report for physical mapping of a major QTL for CBB resistance in common bean.

Introduction
BAC library is stable, rarely chimeric, and easier to manipulate, which makes it popular with researchers (Shizuya et al. 1992). Common bean (*Phaseolus vulgaris* L.), a food legume crop with the smallest genome (about 600 Mb), is very important because it serves as the major plant protein source for people in developing countries. There is an international effort to work on phaseomics (http://www.phaseolus.net/). Its smaller genome size and fewer repetitive sequences attract researchers' efforts to study its important traits using genomics. BAC libraries have been constructed using varieties from common bean and lima bean (*Phaseolus lunatus*). Common bean lines involved are Sprite, BAT98, G02771, and G21245. BAC clones from Sprite hybridizing with markers flanking the nuclear fertility restorer gene, Fr (Vanhouten et al. 1999), an anthracnose resistance gene *Co-4*^{2} (Melotto et al. 2001), and the *I* gene conditioning bean common mosaic virus resistance (Vallejos et al. 2006) have been identified. The arcelin-phytohemagglutinin-α-amylase (APA) families of seed proteins were analyzed by studying four BAC libraries constructed from cultivars with different genotypes, (Kami et al. 2006). A major QTL conditioning common bacterial blight (CBB) resistance on chromosome 1 of common bean is found in HR45 and HR67. It is one of the major sources of CBB resistance in breeding programs worldwide. Its genomic analysis will facilitate its usefulness in genetic studies and breeding programs. The objectives of this research are to: 1) screen BAC pools using tightly linked markers, 2) map this CBB resistance QTL physically using BAC clones.

Materials and Methods
BAC library screening: DNA was extracted from mixed clone cultures (40 µl per clone) of plate pools (PPs), column pools (CPs) and row pools (RPs). All four markers tightly linked to this major CBB resistance QTL were used to identify positive PP, CP and RP. All combinations of target PP, CP and RP from each marker were screened to identify all single target BAC clones of that marker.

BAC fingerprinting and contig assembly: DNA from each BAC clone was digested using *Hin*dIII and run on 0.8% agarose gel. The standard band size from each gel was estimated using Kodak Digital Science 1D Image Analysis Software (Rochester, USA) and then used to set up the standard file for Image 3.10 (http://sanger.ac.uk/Software/Image). The vector band was excluded from the data. Then the output files were further analyzed using FPC 4.8 (Soderlund 2000) to assemble the contigs.

BAC end sequencing and primer design: Forward and reverse BAC ends of most single positive BAC clone were sequenced. The primers were designed using Primer 3 (http://frodo.wi.mit.edu/cgi-bin/primer3) and were used to confirm those overlapping from the BAC digestion.

Results

The insert size distribution: *Not*I digestion of 100 random clones from the library indicated that all of the clones analyzed have an insert and the insert size averaged at 107 kb with a range from 30 kb to 280 kb. Based on the bean genome size of about 637 Mb, the HR45 BAC library contains about 5.7 haploid genome equivalents. Among 100 random clones, 65% do not have endogenous *Not*I sites, 26% have one *Not*I site and 9% have two or three *Not*I sites. Because leaf nuclei were used as the source for high-molecular-weight DNA, contamination of the library with organelle sequences should be low (Vanhouten et al. 1999).

Use of markers to screen the BAC pools: All four molecular markers tightly linked to this major CBB resistance QTL were used to screen the PPs, CPs and RPs. Single BAC clones were confirmed using PCR. Two, four, five, and eight BAC clones gave positive results to UBC420, PV-tttc001, STS333 and STS183, respectively.

BAC contig assembling: All 17 target BAC clones were digested with HindIII and the band were analysed and the contigs were assembled. There are six BACs on the minimum tiling path with a size of about 800 kb. This is subject to further confirmation.

BAC end sequencing and primer design: Both ends of target BAC clones were sequenced and the primers were designed to amplify a band from 350 to 600 bp. The polymorphic markers were mapped back to the QTL region.

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